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I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003903387 for a patent by SIRTEX MEDICAL LIMITED as filed on 02 July 2003.

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Thirteenth day of July 2004

J. Billingsley

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ORIGINAL

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: "Combination Therapy for Treatment of Neoplasia"

The invention is described in the following statement:

"COMBINATION THERAPY FOR TREATMENT OF NEOPLASIA"

FIELD OF THE INVENTION

The present invention concerns an unexpected synergistic combination of known antineoplastic therapies, which provides unexpectedly greater efficacy than either therapy alone in the treatment of neoplasia. Accordingly, the present invention provides a method that has utility in the treatment of various forms of cancer and tumours, but particularly in the treatment of primary liver cancer and secondary liver cancer deriving from the gastrointestinal tract, and more specifically secondary liver cancer deriving from colorectal cancer. It is to be understood that the selective internal radiation therapies described herein should not be limited to radioactive microparticles, but may be extended to any radioactive particles that are suitable for use in the treatment methods described herein.

The invention further provides a synergistic combination of antineoplasia agents, comprising an effective antineoplastic amount of oxaliplatin and an amount of radionuclide-doped microparticles suitable for selective internal radiation therapy (SIRT) to effectively treat a neoplastic growth. Preferentially, oxaliplatin chemotherapy is combined with 5-fluorouracil and leucovorin to enhance the chemotherapeutic effect.

The invention also provides for the use of effective amounts of oxaliplatin and an amount of radionuclide-doped microparticles suitable for SIRT to effectively treat a neoplastic growth in the preparation of a medicament for the treatment of neoplasia generally and in particular primary liver cancer, secondary liver cancer deriving from the gastrointestinal tract, and more specifically secondary liver cancer deriving from colorectal cancer. Also, cancer of the brain, cancer of the kidney, cancer in other soft tissues, and bone sarcomas.

BACKGROUND ART

Neoplasia is now the second leading cause of death in the United States and is a disease characterized by an abnormal proliferation of cell growth known as a neoplasm. Neoplasms may manifest in the form of a leukaemia or a tumour, and

may be benign or malignant. Malignant neoplasms, in particular, can result in a serious disease state, which may threaten life. Significant research efforts and resources have been directed toward the elucidation of antineoplastic measures, including chemotherapeutic and radiotherapeutic agents, which are effective in treating patients suffering from neoplasia. Effective antineoplastic agents include those that inhibit or control the rapid proliferation of cells associated with neoplasms, those that effect regression or remission of neoplasms, and those that generally prolong the survival of patients suffering from neoplasia. Successful treatment of malignant neoplasia, or cancer, requires elimination of all malignant cells, whether they are found at the primary site, or whether they have extended to local-regional areas or have metastasized to other regions of the body.

Of the vast forms of malignant neoplasms colorectal cancer is one of the commonest. The liver is a dominant site of metastatic spread as a result of the portal venous drainage of the gut and is the main cause of death in these patients (Gilbert J, *et al.* (1984) Brit. J Surgery. 71, 203-205). Treatment of such disease states is usually achieved with one or a combination of three therapies: surgery, chemotherapy and radiotherapy.

Surgery involves the bulk removal of diseased tissue. When tumour growth is recognized, excision of the tumour mass by surgery is regarded as the therapy of choice. So, for example, in a minority of patients with liver metastases some form of local ablation, such as surgical resection, cryotherapy or radiofrequency ablation can offer the potential for long-term cure. However, this approach, while producing very satisfactory results as a general measure, is effective only for patients with tumours at an early stage of development. It cannot be used in, for example, the liver where the vast majority of the liver is covered with disseminated neoplastic conditions. Regardless of the developmental stage of the neoplastic mass, therapy through excision is frequently undesirable due to the possibility of missing related growths metastasized to a remote site, the physical scarring left by frequently radical surgical technique, and the risks commonly associated with surgery of any type.

Chemotherapy involves the disruption of cell replication or cell metabolism. It is used most often in the treatment of leukaemia, as well as liver, breast, lung, and testicular neoplasms. In recent years, various excellent antineoplastic compositions have been introduced into use for the chemotherapy with progressively improved results. Chemotherapeutic effects so far achieved nevertheless still remain temporary and are not always satisfactory in completely inhibiting the proliferation of neoplastic tissues and enabling patients to survive a long period of time.

The major classes of chemotherapeutic agents include alkylating agents, antimetabolites and antagonists, and a variety of miscellaneous agents (see Haskell, C. M., ed., (1995) and Dorr, R. T. and Von Hoff, D. D., eds. (1994)).

The classic alkylating agents are highly reactive compounds that have the ability to substitute alkyl groups for the hydrogen atoms of certain organic compounds. The damage they cause interferes with DNA replication and RNA transcription. The classic alkylating agents include mechlorethamine, chlorambucil, melphalan, cyclophosphamide, ifosfamide, thiotepa and busulfan.

Another alkylating agent is Oxaliplatin (OXA). OXA contains a platinum atom which is complexed with oxalate and diaminocyclohexane. Although the exact mechanism of action of OXA is yet not fully understood, it is believed to inhibit DNA synthesis. The primary use of OXA is in colorectal cancer. However, it may also be used to treat other cancers such as breast, gastric, lung, pancreatic and prostrate cancers. Side effects associated with the use of OXA include numbness or tingling in hands and feet due to its effect on the nerve endings; temporary reduction in bone marrow function, resulting in anaemia, risk of bruising or bleeding, nausea and diarrhoea. Less common side effects include laryngeal spasm, allergic reactions, such as skin rashes and itching, and mouth ulcers.

The antimetabolites are structural analogues of normal metabolites that are required for cell function and replication. They typically work by interacting with cellular enzymes. Among the many antimetabolites that have been developed and clinically tested are methotrexate, 5-fluorouracil (5-FU), floxuridine,

cytarabine, 6-mercaptopurine, 6-thioguanine, deoxycoformycin, fludarabine, 2-chlorodeoxyadenosine, and hydroxyurea.

The compound 5-FU is possibly the most widely used antineoplastic drug in the world. 5-FU has been used clinically in the treatment of malignant tumours and cancer, including, for example, carcinomas, sarcomas, skin cancer, cancer of the digestive organs and liver, and breast cancer (A Comprehensive Treatise, 5, 327, Prenum Press, Cancer Res., 18, 478 (1958), Gastroenterology, 48, 430 (1965), Cancer Treat, Rep., 62, 533 (1987)). 5-FU, however, causes serious adverse reactions such as nausea, alopecia, diarrhoea, stomatitis, leukocytic thrombocytopenia, anorexia, pigmentation, and edema (Pharmacological Principles of Cancer Treatment, 195 (1982)). Further, as 5-FU is highly toxic, it is sometimes impossible to administer the compound over a prolonged period of time and therefore to achieve the desired curing effect.

Leucovorin (i.e. (6R,S)-5-formyl-tetrahydrofolate) has been available commercially for decades for the treatment of folic acid deficiency states (The Pharmacologic Basis of Therapeutics, 4th ed. (Goodman et al., eds.) The MacMillan Co., Toronto, pp. 1431-44 (1970)). In 1982, the first clinical reports of the usefulness of leucovorin as a modulator of 5-FU in antineoplastic treatment appeared (Machover *et al.*, (1982) Cancer Treat. Rep, 66, 1803-07). Leucovorin (LV) addition to 5-FU appeared to approximately double response rates in patients with gastrointestinal neoplasms. This result was confirmed in several subsequent studies (see Grem *et al.* (1987), Cancer Treat. Rep. 71:1249-64). Currently, LV addition to 5-FU therapy is community standard practice in the United States.

Combination of the chemotherapy agents OXA, 5-FU and LV is now considered a standard treatment in unresectable rectal cancer patients. In addition, OXA, 5-FU and LV has been used in combination with radiotherapy in the treatment of rectal cancer (Carraro S *et al.*, In J Radiat Oncol Bio Phys 2002, 1:54(2):397-402).

While the combination of such drugs has vastly improved chemotherapy regimens there are still significant problems with the use of such agents. Among

the problems currently associated with the use of such agents to treat neoplastic growth are the high doses of agent required; toxicity toward normal cells, i.e., lack of selectivity; immunosuppression; second malignancies; and drug resistance.

Another side effect associated with present day therapies is the toxic effect of the chemotherapeutic on the normal host tissues that are the most rapidly dividing, such as the bone marrow, gut mucosa and cells of the lymphoid system. The agents also exert a variety of other adverse effects, including neurotoxicity; negative effects on sexuality and gonadal function; and cardiac, pulmonary, pancreatic and hepatic toxicities; vascular and hypersensitivity reactions, and dermatological reactions.

The clinical usefulness of a chemotherapeutic agent may also be severely limited by the emergence of malignant cells resistant to that drug. A number of cellular mechanisms are probably involved in drug resistance, e.g., altered metabolism of the drugs, impermeability of the cell to the active compound or accelerated drug elimination from the cell, altered specificity of an inhibited enzyme, increased production of a target molecule, increased repair of cytotoxic lesions, or the bypassing of an inhibited reaction by alternative biochemical pathways. In some cases, resistance to one drug may confer resistance to other, biochemically distinct drugs. In this respect amplification of the gene encoding thymidylate synthase is related to resistance to treatment with 5-fluoropyrimidines.

In summary, chemotherapy has not made a dramatic impact on the treatment of neoplastic growths. Certain drugs and biologicals have shown considerable activity in various studies, but their effects are negated by numerous problems and disadvantages. Patients usually develop resistance to each specific chemotherapy drug and so ultimately all chemotherapy drugs fail leaving nowhere to go, and often even the initial response to the first treatment with a drug that the patient has not had before, is a small response.

Radiotherapy has been used as an alternative to chemotherapy and usually relies on treatment through external beam technologies or more recently through locally administering radioactive materials to patients with cancer as a form of

therapy. In some of these, the radioactive materials have been incorporated into small particles, seeds, wires and similar related configurations that can be directly implanted into the cancer. When radioactive particles are administered into the blood supply of the target organ, the technique has become known as Selective Internal Radiation Therapy (SIRT). Generally, the main form of application of SIRT has been its use to treat cancers in the liver.

There are many potential advantages of SIRT over conventional, external beam radiotherapy. Firstly, the radiation is delivered preferentially to the cancer within the target organ. Secondly, the radiation is slowly and continually delivered as the radionuclide decays. Thirdly, by manipulating the arterial blood supply with vasoactive substances, it is possible to enhance the percentage of radioactive particles that go to the cancerous part of the organ, as opposed to the healthy normal tissues. This has the effect of preferentially increasing the radiation dose to the cancer while maintaining the radiation dose to the normal tissues at a lower level (Burton, M.A. *et al.* (1988) Europ. J. Cancer Clin. Oncol. 24(8), 1373-1376).

When microparticles or other small particles are administered into the arterial blood supply of a target organ, it is desirable to have them of a size, shape and density that results in the optimal homogeneous distribution within the target organ. If the microparticles or small particles do not distribute evenly, and as a function of the absolute arterial blood flow, then they may accumulate in excessive numbers in some areas and cause focal areas of excessive radiation.

For radioactive particulate material to be used successfully for the treatment of neoplastic growth, the radiation emitted should be of high energy and short range. This ensures that the energy emitted will be deposited into the tissues immediately around the particulate material and not into tissues that are not the target of the radiation treatment. In this treatment mode, it is desirable to have high energy but short penetration beta-radiation, which will confine the radiation effects to the immediate vicinity of the particulate material. There are many radionuclides that can be incorporated into microparticles that can be used for SIRT. Of particular suitability for use in this form of treatment is the unstable

isotope of yttrium (Y-90). Yttrium-90 decays with a half-life of 64 hours, while emitting a high energy pure beta radiation. However, other radionuclides may also be used in place of Y-90 of which the isotopes of holmium, samarium, iodine, iridium, phosphorus, rhenium are some examples.

The technique of SIRT has been previously reported (see, for example, Chamberlain M, *et al* (1983) Brit. J. Surg., 70: 596-598; Burton MA, *et al* (1989) Europ. J. Cancer Clin. Oncol., 25, 1487-1491; Fox RA, *et al* (1991) Int. J. Rad. Oncol. Biol. Phys. 21, 463-467; Ho S *et al* (1996) Europ J Nuclear Med. 23, 947-952; Yorke E, *et al* (1999) Clinical Cancer Res, 5 (Suppl), 3024-3030; Gray BN, *et al.* (1990) Int. J. Rad. Oncol. Biol. Phys, 18, 619-623). Treatment with SIRT has been shown to result in high response rates for patients with neoplastic growth in particular with colorectal liver metastases (Gray B.N. *et al* (1989) Surg. Oncol, 42, 192-196; Gray B, *et al.* (1992) Aust NZ J Surgery, 62, 105-110; Gray B N *et al.* (2000) GI Cancer, 3(4), 249-257; Stubbs R, *et al* (1998) Hepato-gastroenterology Suppl II, LXXVII). Other studies have shown that SIRT therapy can also be effective in causing regression and prolonged survival for patients with primary hepatocellular cancer (Lau W, *et al* (1994) Brit J Cancer 70, 994-999; Lau W, *et al.* (1998) Int J Rad Oncol Biol Phys. 40, 583-592). Although SIRT is effective in controlling the liver disease, it has no effect on extra-hepatic disease.

Recently, clinicians have tried to improve the response of cancer patients by combining two or more anti-tumour therapies into a single therapeutic regimen. By combining two or more therapies, most often with different mechanisms of action, the clinician is able to both increase the therapeutic index of the individual treatments, and at the same time reduce the toxic effects to the patient.

One example of such combination therapy are the randomised clinical trials carried our by Gray *et al* where they compare treatment of floxuridine either with or without the addition of a single dose of radioactive microparticles (Gray et al (2001) Annals of Oncology 12: 1711-1720). Results from these studies have shown that the addition of radioactive microparticles increased the response rate from 17.6% to 44% and the time to disease progression from 9.7 months to 15.9

months. An important finding from this trial was that although most patients eventually succumbed to their disease, the liver metastases were not the primary cause of death for most patients treated with SIRT.

Combination therapies now being tested use drugs with dissimilar mechanisms of action, based on the rationale that targeting two independent pathways will result in enhanced cytotoxicity, whether additive or synergistic. The results of these experiments are entirely unpredictable as the use of two entirely different therapies usually means that each therapy works independent of the other and thus would not be expected to interact to improve the other.

Combination treatments that expose tumours to high concentrations of antineoplastic drugs and other anti-tumour agents would be an advance in therapy for cancer in the liver. Moreover, it would be desirable to have a method that substantially reduces the disease progression in a patient. There is described herein a process which provides such advantages.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides a method of treating neoplasia in a subject in need of treatment, by administering to the subject an amount of OXA effective to treat a neoplasia, in combination with SIRT, wherein a synergistic antineoplastic effect results. Although OXA may be the only chemotherapeutic agent employed in the method, it will be appreciated that other chemotherapeutic agents may be used in the method. Preferably each of 5-FU and or LV, are included either in combination or individually. Other chemotherapeutic agents that may be employed in the method either in addition to 5-FU and or LV include systemic chemotherapy drugs such as irinotecan or capecitabine. Further the method may also include a step of treating the patient with anti-angiogenesis factors, ie drugs that inhibit tumours vascularising. Preferably, the method is used for treating a patient with colorectal liver metastases.

The present invention further provides a synergistic combination of antineoplastic agents, comprising an effective antineoplastic amount of OXA and an amount of radionuclide-doped microparticles suitable for use in SIRT for treatment of a

neoplastic growth. Preferably, the combination is prepared for use in treating a patient with colorectal liver metastases.

The invention also relates to pharmaceutical composition comprising an effective antineoplastic amount of OXA and an amount of radionuclide-doped microparticles suitable for use in SIRT for treatment of a neoplastic growth. Preferably, the pharmaceutical composition is prepared for use in treating a patient with colorectal liver metastases. In addition to the pharmaceutical composition including OXA it may include one or more alternate chemotherapeutic agents and or anti-angiogenesis factors. Such agents will include but will not be limited to 5-FU, LV, irinotecan or capecitabine.

The invention still further relates to use of an effective antineoplastic amount of OXA and an amount of radionuclide-doped microparticles suitable for use in SIRT, for manufacture of a medicament for killing neoplastic cells in a subject having neoplastic cells. Preferably, the medicament is prepared for use in treating a patient with colorectal liver metastases. In addition the medicament manufactured according to this aspect of the invention may also include one or more alternate chemotherapeutic agents and or anti-angiogenesis factors. Such agents will include but will not be limited to 5-FU, LV, irinotecan or capecitabine.

Other aspects and advantages of the invention will become apparent to those skilled in the art from a review of the ensuing description.

DETAILED DISCLOSURE OF THE INVENTION

General

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variation and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in the specification, individually or collectively, and any and all combinations or any two or more of the steps or features.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally equivalent products, compositions and methods are clearly within the scope of the invention as described herein.

All references cited, including patents or patent applications are hereby incorporated by reference. No admission is made that any of the references constitute prior art.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout.

Description of Preferred Embodiments

Surprisingly applicants have found that the co-administration of systemic chemotherapy and SIRT to a subject, potentates the radiation from SIRT, and also has an effect on extra-hepatic disease.

Accordingly, the present invention provides a method of treating neoplasia in a subject in need of treatment, by administering to the subject an amount of OXA effective to treat a neoplasia, in combination with SIRT, wherein a synergistic antineoplastic effect results.

Although OXA may be the only chemotherapeutic agent employed in the method, it will be appreciated that other chemotherapeutic agents may be used in the method. Preferably each of 5-FU and or LV; are included either in combination or individually. For ease of description the following disclosure is framed in terms of using OXA in combination with 5-FU and LV as this is a common combination used for the treatment of malignant neoplasias. The present invention should not be read as being limited to only the use of such a combination in the method, but includes only the use of OXA in the method or the use of OXA and 5-FU or the

use of OXA and LV or the use of any of these combinations with other chemotherapeutic agents.

Other chemotherapeutic agents that may be employed in the method either in addition to 5-FU and or LV include systemic chemotherapy drugs such as irinotecan or capecitabine. Further, the method may also include a step of treating the patient with anti-angiogenesis factors, i.e. drugs that inhibit tumours vascularising. Preferably, the method is used for treating a patient with colorectal liver metastases.

The present invention provides a method of treating malignant neoplasia. Neoplasias for which the present invention will be particularly useful include, without limitation, primary liver cancer and secondary liver cancer deriving from the gastrointestinal tract, and more specifically secondary liver cancer deriving from colorectal cancer. Also, cancer of the brain, cancer of the kidney, cancer in other soft tissues, and bone sarcomas.

In the method of the present invention, 5-FU, LV and OXA is administered to a subject in combination SIRT, such that a synergistic antineoplastic effect is produced. A "synergistic antineoplastic effect" refers to a greater-than-additive antineoplastic effect that is produced by a combination of chemotherapeutic drugs and SIRT, which exceeds that which would otherwise result from individual therapy associated with either therapy alone. Treatment with 5-FU, LV and OXA in combination with SIRT unexpectedly results in a synergistic antineoplastic effect by providing greater efficacy than would result from use of either of the antineoplastic agents alone.

In the method of the present invention, administration of 5-FU, LV and OXA "in combination with" SIRT refers to co-administration of the three antineoplastic treatments. Co-administration may occur concurrently, sequentially, or alternately. Concurrent co-administration refers to administration of 5-FU, LV and OXA and SIRT at essentially the same time. For concurrent co-administration, the courses of treatment with 5-FU, LV and OXA and with SIRT may also be run simultaneously. For example, a single, combined formulation of

5-FU, LV and OXA, in physical association with SIRT, may be administered to the subject.

In the method of the present invention, 5-FU, LV and OXA therapy and SIRT also may be administered in separate, individual treatments that are spaced out over a period of time, so as to obtain the maximum efficacy of the combination. When spaced out over a period of time, administration of 5-FU, LV and OXA is preferably given to a patient for a period of time such as 1 to 10 days, but more preferably about 3 to 5 days following which SIRT is applied. This cycle may be repeated as manner times as necessary and as long as the subject is capable of receiving said treatment.

As used herein "treatment" includes:

(i) preventing a disease, disorder or condition from occurring in an subject which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;

(ii) inhibiting the disease, disorder or condition, i.e., arresting its development; or

(iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

In the method of the present invention, neoplasia is treated in a subject in need of treatment by administering to the subject an amount of a combination of 5-FU, LV and OXA effective to treat a neoplasia in combination with a sufficient amount of SIRT to treat a neoplasia, wherein a synergistic antineoplasia effect results.

The subject is preferably a mammal and is most preferably a human.

5-FU, LV and OXA chemotherapy

In the method of the present invention, an amount of 5-FU, LV and OXA that is "effective to treat the neoplasia" is an amount that is effective to ameliorate or minimize the clinical impairment or symptoms of the neoplasia, in either a single or multiple dose of 5-FU, LV and OXA when combined with SIRT. For example, the clinical impairment or symptoms of the neoplasia may be ameliorated or minimized by diminishing any pain or discomfort suffered by the subject; by

extending the survival of the subject beyond that which would otherwise be expected in the absence of such treatment; by inhibiting or preventing the development or spread of the neoplasm; or by limiting, suspending, terminating, or otherwise controlling the maturation and proliferation of cells in the neoplasm. Notably, the amounts of 5-FU, LV and OXA effective to treat neoplasia in a subject in need of treatment will vary depending on the type of SIRT used, as well as the particular factors of each case, including the type of neoplasm, the stage of neoplasia, the subject's weight, the severity of the subject's condition, and the method of administration. These amounts can be readily determined by the skilled artisan.

5-FU, LV and OXA treatment according to the present invention may be administered to a subject by known procedures, including, but not limited to, oral administration, parenteral administration (e.g., intramuscular, intraperitoneal, intravascular, intravenous, or subcutaneous administration), and transdermal administration. Preferably, the 5-FU, LV and OXA agents are administered parenterally.

The formulations may be presented in unit or multi-dose containers, such as sealed ampoules or vials. Moreover, the formulations may be delivered by any mode of injection, including, without limitation, epifascial, intracapsular, intracutaneous, intramuscular, intraorbital, intraperitoneal (particularly in the case of localized regional therapies), intraspinal, intrasternal, intravascular, intravenous, parenchymatous, or subcutaneous, and intratumoral.

SIRT Therapy

According to the invention the person skilled in the art will appreciate that SIRT may be applied by any of a range of different methods, some of which are described in US patents 4789501, 5011677, 5302369, 6296831, 6379648, or WO applications 200045826, 200234298 or 200234300. Accordingly administration of radionuclide doped microparticles may be by any suitable means, but preferably by delivery to the relevant artery. For example in treating liver cancer, administration is preferably by laparotomy to expose the hepatic artery or by insertion of a catheter into the hepatic artery via the femoral, or

brachial artery. Pre or co-administration of another agent may prepare the tumour for receipt of the particulate material, for example a vasoactive substance, such as angiotension-2 to redirect arterial blood flow into the tumour. Delivery of the particulate matter may be by single or multiple doses, until the desired level of radiation is reached.

The radionuclide doped microparticles need not be limited to any particular form or type of microparticle. So, for example, the radionuclide doped microparticles suitable for use in the invention may comprise any material capable of receiving a radionuclide such as through impregnation, absorbing, coating or more generally bonding the particles together.

In one particular form of the invention the microparticles are prepared as polymeric particles. In another form of the invention the microparticles are prepared as ceramic particles (including glass). In another, they are prepared from chitosan.

Where the microparticles are prepared as polymeric matrix they will preferably have a stably incorporated radionuclide. More preferably the radionuclide will be incorporated by precipitation of the radionuclide as a salt. A description of such particles including methods for there production and formulation as well as there use is provided in co-owned European application number 200234300, of which the teachings therein are expressly incorporated herein by reference.

Where the microparticles are ceramic particles (including glass) the selected particles will usually possess the following properties:

(1) the particles will generally be biocompatible, such as calcium phosphate-based biomedical ceramics or glass.

(2) the particles will generally comprise a radionuclide that preferably has sufficiently high energy and an appropriate penetration distance, which are capable of releasing their entire energy complement within the tumour tissue to effectively kill the cancer cells and to minimize damage to adjacent normal cells or to attending medical personnel. The level of radiation activity of the ceramic or glass will be selected and fixed based upon the need for therapy given the particular cancer involved and its

level of advancement. The ideal half-life of the radionuclides is somewhere between days and months. On the one hand, it is impractical to treat tumours with radionuclides having too short a half-life, this characteristic limiting therapy efficiency. On the other hand, in radiotherapy it is generally difficult to trace and control radionuclides having a long half-life.

(3) Third, the particles must be of a suitable size. The size of the particles for treatment depends upon such variables as the surface area of the tumour, capillary permeability, and the selected method of introduction into the tumour (i.v. versus implant by surgical operation).

(4) Fourth, some ceramic processes involve inclusion of extraneous substances as contaminants that might produce undesired radionuclides. Should these be well taken care of, the size of the particles can then be controlled by granulation and meshing.

There are many processes for producing small granular ceramic or glass particles. One of these involves the introduction of small amounts of the ceramic particles passing through a high-temperature melting region. Ceramic spherules are yielded by surface tension during melting. After the solidification, condensation, collection and sorting processes, ceramic spherules of various sizes can be obtained. The particle size of ceramic spheroid can be controlled by the mass of granules introduced into the high-temperature melting region or can be controlled by collecting spheroids of various sizes through the selection of sedimentary time during liquid-sedimentation.

The ceramic or glass materials for preparing those particles can be obtained commercially or from ultra-pure ceramic raw materials if the commercial products do not meet specifications for one reason or another. The ceramic or glass particles for radiation exposure in this invention can be yielded by traditional ceramic processes, which are well known by those skilled in this art. The ceramic processes such as solid-state reaction, chemical co-precipitation, sol-gel, hydrothermal synthesis, glass melting, granulation, and spray pyrolysis can be applied in this invention for the production of specific particles.

The microparticles of the invention be they polymer or ceramic based can be separated by filtration or other means known in the art to obtain a population of microparticles of a particular size range that is preferred for a particular use. The size and shape of the microparticles is a factor in the distribution and drug delivery in the tissues.

The radionuclide which is incorporated into the microparticles in accordance with the present invention is preferably yttrium-90, but may also be any other suitable radionuclide such as holmium, samarium, iodine, phosphorous, iridium and rhenium.

The amount of microparticles used in the method and which will be required to provide effective treatment of a neoplastic growth will depend substantially on the radionuclide used in the preparation of the microparticles. By way of example, an amount of yttrium-90 activity that will result in an inferred radiation dose to the normal liver of approximately 80 Gy may be delivered. Because the radiation from SIRT is delivered as a series of discrete point sources, the dose of 80 Gy is an average dose with many normal liver parenchymal cells receiving much less than that dose. Alternate doses of radiation may be delivered depending on the disease state and the physician's treatment needs. Such variation of radiation doses by altering the amount of microparticles used will be something that a skilled artisan will know how to determine.

The term microparticle is used in this specification as an example of a particulate material, it is not intended to limit the invention to microparticles of any particular shape or configuration. A person skilled in the art will however appreciate that the shape of the particulate material while preferably be without sharp edges or points that could damage the patients arteries or catch in unintended locations. Preferably the particulate material is substantially spherical, but need not be regular or symmetrical in shape.

In a highly preferred form of the invention there is provided a method for treating neoplasia in a subject in need of treatment, by administering an effective antineoplastic amount of 5-FU, LV and OXA as described above in combination with an amount of radionuclide-doped microparticles suitable for use in SIRT for

treatment of a neoplastic growth, wherein a synergistic antineoplasia effect results.

In addition to the identified chemotherapeutic agents and radionuclide doped microparticles the invention may also include an effective treatment of immunomodulators and other agents as part of the therapy. Illustrative immunomodulators suitable for use in the invention are alpha interferon, beta interferon, gamma interferon, interleukin-2, interleukin-3, tumour necrosis factor, granulocyte-macrophage colony stimulating factors, and the like. Also anti-angiogenesis agents such as Avatin (Genentech) which impedes blood supply to tumours.

The present invention further provides a synergistic combination of antineoplastic agents, comprising an effective antineoplastic amount of 5-FU, LV and OXA and an amount of radionuclide-doped microparticles suitable for use in SIRT for treatment of a neoplastic growth. Preferably, the combination is prepared for use in treating a patient with colorectal liver metastases.

The invention also relates to pharmaceutical composition comprising an effective antineoplastic amount of 5-FU, LV and OXA and an amount of radionuclide-doped microparticles suitable for use in SIRT for treatment of a neoplastic growth. Preferably, the pharmaceutical composition is prepared for use in treating a patient with colorectal liver metastases.

The invention still further relates to use of an effective antineoplastic amount of 5-FU, LV and OXA as described above and an amount of radionuclide-doped microparticles as described above suitable for use in SIRT, for manufacture of a medicament for killing neoplastic cells in a subject having neoplastic cells. Preferably, the medicament is prepared for use in treating a patient with colorectal liver metastases.

The invention yet further relates to the use of an effective antineoplastic amount of 5-FU, LV and OXA as described above and an amount of radionuclide-doped microparticles as described above suitable for use in SIRT, for manufacture of a kit for killing neoplastic cells in a subject having neoplastic cells.

BEST MODE(S) FOR CARRYING OUT THE INVENTION

Further features of the present invention are more fully described in the following non-limiting Examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention. It should not be understood in any way as a restriction on the broad description of the invention as set out above.

Patients: Six patients with colorectal liver metastases either with or without extra-hepatic metastases were enrolled in this study. Patients were between 45 and 70 years of age, had histologically proven adenocarcinoma of the colorectum, unequivocal CT scan evidence of liver metastases that could not be treated by resection or any locally ablative technique.

Patients received systemic chemotherapy (5-FU, LV and OXA) with the addition of a single administration of SIR-Spheres® (SIRTeX Medical Ltd). All patients had multiple bi-lobar liver metastases and were reviewed in a surgical oncology unit to confirm that the metastases were so advanced that they were unable to be treated by any form of local ablation.

Investigations: All patients underwent a pre-treatment spiral CT scan of the whole abdomen and either a CT scan of the chest or chest X-ray and blood tests to assess haematologic, renal and liver function and serum CEA.

Patients treated with SIRT underwent a trans-femoral hepatic angiogram to assess the arterial anatomy of the liver and to plan the subsequent administration of SIR-Spheres®. Patients treated with SIRT also underwent a nuclear medicine planar and SPECT scans to estimate the amount of SIR-Spheres® that would pass through the liver and lodge in the lungs. This was performed by injecting technetium-99 labelled macro-aggregated albumin (MAA) into the hepatic artery at the time of the angiogram and measuring the radioactivity in the liver and lungs using a gamma camera. Areas of interest were drawn around the liver and lungs and the percentage of the MAA that lodged in the lungs was determined as a fraction of the total amount of MAA in both lungs and liver. This was recorded as a 'percentage lung break-through' in

order to decide whether to reduce the amount of yttrium-90 activity to administer to the patient. Previous experiments had shown that a lung break-through percentage of >13% might result in radiation pneumonitis and should be accompanied by a reduction in the amount of yttrium-90 activity administered to the patient (Ho S *et al* (1996) Europ J Nuclear Med. 23, 947-952). This technique has been shown to be a reliable method for determining the subsequent distribution of SIR-Spheres®.

Patients were followed after trial entry with three monthly clinical evaluations and quality of life assessment (QoL), three-monthly CT scans of the abdomen were also carried out as were either a plain X-ray or CT scan of the chest. Further, monthly regular serologic tests of haematologic, liver and renal function and CEA were taken. Patients found to have obtained a complete (CR) or partial (PR) response on CT scan had a second confirmatory CT scan at not less than 4 weeks after the initial scan that showed the response.

Recording of Response and Toxicity: Response was determined using RECIST criteria (Therasse P *et al* (2000) J Natl Cancer Inst 92, 205-216). The RECIST criteria were developed with particular application for reporting the results of phase 2 trials and result in very similar response outcomes as the conventional WHO method.

Toxicity was recorded on all patients using standard UICC recommendations for grading of acute and subacute toxicity criteria.

Protocol Treatment: Patients were treated with a combination of Oxaliplatin, 5-Fluorouacil, Leucovorin (FOLFOX-4) and SIR-spheres. Oxaliplatin 30mg/m^2 or 60mg/m^2, dependent on treatment group, was administered on day 1 of each cycle. Leucovorin 200 mg/m^2 followed by 5-fluorouracil 400 mg/m^2 as IV bolus and 600 mg/m^2 5-Fluorouracil as 22-h continuous infusion were administered on days one and two of each cycle. Chemotherapy cycles were repeated at two weekly intervals and continued in patients until evidence of unacceptable toxicity, patient request or disease progression. Patients received a maximum of 12 cycles of protocol chemotherapy.

Patients received a single dose of SIR-Spheres® that was administered on either day two or day three of the first cycle of chemotherapy. The SIR-Spheres® was administered into the hepatic artery via a trans-femoral catheter that was placed under local anaesthetic. In patients where there was more than one hepatic artery supplying blood to the liver, the catheter was repositioned during administration and the total dose of SIR-Spheres® was divided into separate aliquots depending on the estimated volume of tumour being supplied by each feeding artery. Patients treated with SIRT were generally kept in hospital overnight and discharged home the following day.

Patients were treated with a dose of SIR-Spheres® that was calculated from the patient's body surface area and the size of the tumour within the liver according to the following equation;

$$\text{Dose of SIR-Spheres}^{®} \text{ in GBq} = (\text{BSA}^{*} - 0.2) + \left(\frac{\%\ tumour\ involvement}{100}\right)$$

* BSA = body surface area measured in square metres

Non-Protocol Treatment: Once protocol treatment ceased, further cancer specific treatment, including non-protocol chemotherapy, was allowed to best manage patient care. All non-protocol cancer specific treatment was recorded in all patients. Other supportive, but not cancer specific treatment was allowed for patient management.

RESULTS

Patients: Three patients (numbered 201002, 201003 and 605001) were treated at the initial Oxaliplatin dose level, 30mg/m^2 and received between 1.3 – 3.2 GBq of Sir-spheres. One patient (605001) has completed chemotherapy as per the protocol (12 cycles), and two patients remain on protocol chemotherapy. Of the initial 3 patients, all have shown evidence of response with some reduction in tumour size on CAT scans. Two patients have demonstrated partial response and one patient has shown stable disease on initial follow-up scan, by RECIST criteria.

Protocol Treatment: Since protocol treatment was well tolerated at the first dose level a further three patients were treated at the higher dose level, 60mg/m^2 Oxaliplatin, and received between 0.9-1.6 GBq of Sir-spheres. Two patients at this dose level have shown response to treatment with reduction in CEA, one patient has yet to be fully assessed but has shown a 26% reduction as per RECIST. At the time of writing all patients in the second treatment group remain on protocol chemotherapy and are being followed to assess toxicity and radiological response to treatment.

Table 1 below details haematological toxicities experienced by patients on protocol treatment. There were no treatment related serious adverse events reported. However, patient 201002 experienced Grade 3 intermittent nausea/vomiting on one day of cycle 1, most likely due to the chemotherapy. Anti-emetics were prescribed to the patient. In addition, patient 201003 experienced Grade 3 diarrhoea/lose stools for about 5 days during Cycle 2. The patient was administered IV fluids and PRN Loperamide during day visits to the hospital. The most likely cause of the diarrhoea was chemotherapy.

Patient ID	Parameter	UICCI Grade
201002	Haemoglobin	2
	Leucocytes	1
201003	Haemoglobin	1
	ALT/AST	1
	Alkaline Phosphate	2
605001	Alkaline Phosphate	2
	Granulocytes	2
	Leucocytes	2
	Haemoglobin	2

Table 1

Haematological toxicities experienced by patients

Dated this SECOND day of JULY 2003.

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